Session 6 Bacteria

Abstracts of Presentations

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Bart Cottyn

QBOL – WP 4: Barcode identification of quarantine bacteria, the QBOL strategy and results

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Within QBOL, barcode sequences have been defined for a group of quarantine bacteria from the combined EU/EPPO lists, comprising the three *Clavibacter michiganensis* subspecies, *Ralstonia solanacearum*, *Xylella fastidiosa* and a selection of *Xanthomonas* pathogens.
The challenges were to compose a relevant collection of quarantine and closely related bacteria, to unravel their taxonomic and pathogenic identity, and to define representative barcode sequence regions.
A barcoding strategy had to be developed. For this it was not possible to use other barcoding of life (BOL) programs, which study existing diversity of eukaryotes, but not of prokaryotes. Moreover, several quarantine bacteria are named as pathovars, an infra-species division that refers to a pathological specialization and does not necessarily correlate with a taxonomic unit. Barcode identification of the quarantine bacteria uses several gene sequences in a step-wise approach following different flows in a decision scheme. The barcoding principle and process will be illustrated for a set of *Xanthomonas* pathovars.

Isabelle Robène-Soustrade

PCR-based assays for detecting *Xanthomonas axonopodis* pv. *allii* in onion seed.

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Bacterial blight of onion is an emerging disease threatening world onion production, and causing damage to other *Allium* crops. The causal agent, *Xanthomonas axonopodis* pv. *allii* (Xaa) has been listed on the EPPO A1 list of pests recommended for regulation as quarantine pests, since 2009. A duplex nested-PCR assay, targeting two markers specific to Xaa has been recently developed (Robène-Soustrade *et al*., 2010). A triplex quantitative real-time PCR assay (Taqman® technology) was developed targeting the same Xaa-specific markers and an internal control chosen in 5.8S rRNA gene from Alliaceae. Xaa strains were detected by the amplification of one or both of the two specific markers. The internal control signal validates both the extraction process and the reaction itself. Several successive steps have to be performed before detection from seed: seed maceration for 48h at 4°C, followed by homogenization of the seed macerate with a stomacher® and DNA extraction using DNeasy® Plant mini kit (Qiagen). This assay is currently being validated following the European standard EN ISO 16140: 2003 and the EPPO standard PM7/98 (1). The performance of Nested-PCR and real-time PCR assays are discussed. These PCR-based tools could be useful for the international sanitary surveillance of seed exchanges.

Reference: Robène-Soustrade, I., Legrand, D., Gagnevin, L., Chiroleu, F., Laurent, A., Pruvost, O. (2010). Appl. Environ. Microbiol. 76, 2697-2703.